

Development of Rapid Colorimetric Assay for Detection of Gluconic Acid Using Iron(α) and Indigo Carmine

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Summary: In this work, a simple and rapid spectrophotometric method, which is based on the fact that Iron(II)-gluconic acid complex as a kind of reducing agent deterioration of indigo carmine dyes, was developed to detect gluconic acid in food. Under the optimal experimental condition, a linear range of 3.6 μ M to 900 μ M was obtained for gluconic acid with a limit of detection of 1.1 μ M. The colorimetric method was rapid and robust with a low cost and can be applied to gluconic acid detection in food samples.

Keywords: Gluconic acid; Iron(II); Indigo carmine; Colorimetric method; Food analysis.

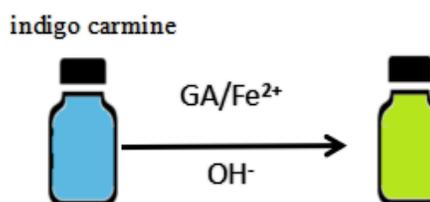
Introduction

Gluconic acid (GA) is mainly employed as an acid flavoring agent, nutrient additive in the food industry and it is often used in the preparation of descaling agent and pH regulator in the chemical industry. It was reported that GA and its δ -lactone, glucolactone have influence on the proliferation of *Bifidobacterium*[1]. As an important food additive, glucolactone is often used as coagulant for milk protein and soybean protein and it can only be converted into gluconic acid to solidify tofu, anti-corrosion and other functions. Based on this, the content of GA is often used to evaluate the quality of glucolactone in the field of food industry. Therefore, the accurate detection and quantification of GA is critical for food and nutrient hygienical field.

Several methods have been employed for the determination of GA in musts and wines including electrochemical[2-6], capillary electrophoresis[7], HPLC[8-12], GC[13], IC[14], FT-IR[15], spectroscopy. However, these methods often suffered from time consuming, low sensitivity or high cost, etc. Hence, it is still desired to develop a new method for the simple, rapid and low-cost detection of GA.

Compared with the previous reports, the colorimetric strategy is convenient, simple and specific. It has been widely used for the detection of various metals such as Cu^{2+} , Hg^{2+} , Ag^{+} [16-18], glucose[19], H_2O_2 [20] and various amino acid such as ascorbic acid, *L*-cysteine, uric acid [21-23], in which the synthesis of various receptor compounds was involved. Unfortunately, it has not been reported for the sensing GA to employ the colorimetric technique. Herein, we proposed a simple, rapid,

reliable and sensitive colorimetric strategy to detect GA in food samples based on indigo carmine discoloration, in which the synthesis of complex receptor compounds is avoid. As illustrated in Scheme 1, under the alkaline condition, indigo carmine dye is bright blue and it becomes greenish-yellow when reduced by the complex of ferric ion and gluconic acid (GA/Fe^{2+}). Based on this, a simple and economical colorimetric strategy is presented for the detection of GA.



Scheme-1: Schematic illustration of the sensing of gluconic acid based on the degradation of indigo carmine.

Experimental

Chemicals and Materials

FeSO_4 , Na_2HPO_4 , NaH_2PO_4 , gluconic acid and indigo carmine were purchased from Aladdin Reagent (Shanghai, China). Zn^{2+} , Mg^{2+} , Ba^{2+} , Mn^{2+} , Ag^{+} , Na^{+} , Fe^{3+} , Cl^{-} , CO_3^{2-} , SO_4^{2-} from Tianjin chemical reagent company (Tianjin, China). Citric acid, *L*-ascorbic acid, and cysteine are from Aladdin Reagent (Shanghai, China). All the ultrapure water from the Milli-Q water purification system (Milipore, Bedford, MA $\geq 18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$).

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Colorimetric Detection of Gluconic acid

In a 10.0 mL microtube, gluconic acid of various concentrations, 400 μL FeSO_4 (0.216 mM), 3 mL PBS (20 mM, pH 11.52) and 2 mL indigo carmine (0.013 mM) were added and the mixture was diluted to the scale using the double deionized water. Then shaken thoroughly until the color does not change and immediately transferred the mixed solution into a 1.0 cm standard quartz cell. Subsequently the absorption of the solutions was recorded at 610 nm using the ultraviolet spectrophotometry (Perkin Elmer, China) after 1 min. For detection of gluconic acid in Lactone tofu, the sample was filtered through filter membrane (15 ~ 20 μm , $\Phi=11$ cm) and the absorption spectra were measured based on the above mentioned procedure.

Results and Discussion

Optimization of the experimental conditions

Effect of pH

The pH value of the sensing system usually has a significant effect on the stability of ferrous hydroxide. Effect of pH value on the absorbance of the sensing system at 610 nm was examined and the results are shown in Fig 1. It can be seen that, the absorbance of the sensing system at 610 nm gradually decreases with the pH value increasing, and reaches the lowest value at pH 11.52. Subsequently, the change of the absorbance of the system gradually leveled off during pH 11.52 and 12.92. This may be because alkaline medium is necessary for the formation of stable ferrous salt complexes. Notably that too strong alkalinity will destroy the stability of the ferrous salts, which will cause the redox effect [24]. Therefore, the pH value of 11.52 was chosen for the further experiments.

Effect of the ratio of GA/Fe^{2+} and indigo carmine

The effects of the concentration ratio of GA/Fe^{2+} complex and indigo carmine on the discoloration of dye were studied. The procedures were the same as the detection of the GA except that the concentration ratio of GA/Fe^{2+} and the indigo carmine varied in the range of 1:1 and 10:1, the result is shown in Fig 2, shown that the absorption of the sensing system decreased with the increasing of the ratio of the GA/Fe^{2+} . When the ratio of GA/Fe^{2+} and the indigo carmine was in the range of 3:1 and 5:1, the absorption of the system was minimum. It indicated that the GA/Fe^{2+} exhibited the strongest reduction capability while the ratio of GA/Fe^{2+} and

the indigo carmine is 3:1 to 5:1. Subsequently, the dye will no longer be reduced as the increasing of the ratio of GA/Fe^{2+} . As such, the ratio of GA/Fe^{2+} complex and indigo carmine of 3.3:1 was chosen for the further experiments.

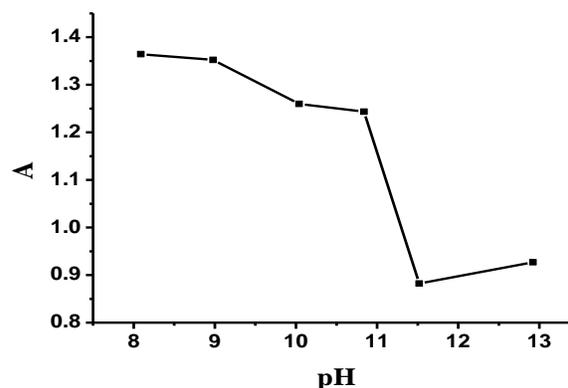
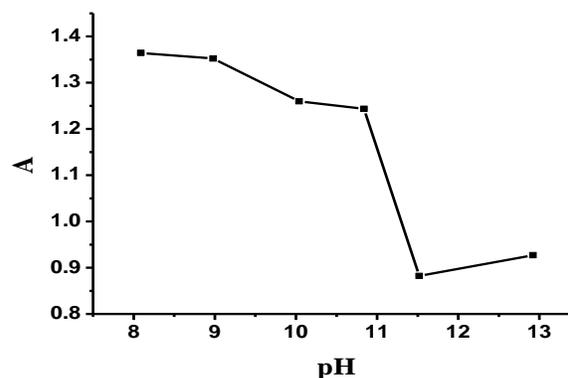


Fig. 1: Absorbance changes of the indigo carmine/ Fe^{2+} (2.6 μM /8.64 μM) sensing system at 610 nm in the presence of GA (360.0 μM) in different pH medium from 8.09 to 12.92.

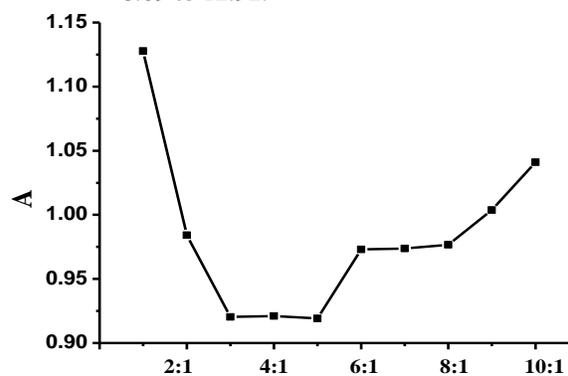


Fig. 2: Effect of the ratio of GA/Fe^{2+} the indigo carmine on the absorption of the sensing system of GA/Fe^{2+} .

Detection of Gluconic acid

Fig 3 (line a) shows the absorption of indigo carmine dye in PBS solution in the presence of ferric ion. As can be seen, indigo carmine exhibited a specific absorption band at 610 nm, which is from the absorption of the π -electron system, produced by $-\text{NH}$ and HSO_3^- group on the benzene ring of dihydroindole sulfonate [25-26]. After addition of GA in the sensing system, there is hypochromic effect on the absorption spectra of indigo carmine with the increasing of concentration of GA (line b to j, Fig 3), accompanied with the color changes of the solution, from light blue to greenish-yellow (Fig 3 inset). The possible mechanism is that ferrous ion and GA formed complex in the alkaline medium, which is a reducing agent that can degrade the indigo carmine to discolor [27]. Indigo carmine may have various degradation products including *o*-nitrobenzoic acid, *o*-aminobenzoic acid, which can break the group $-\text{C}=\text{C}-$, leading the absorption decreasing of the sensing system.

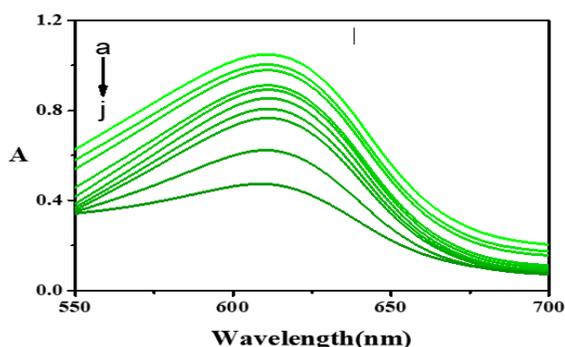


Fig. 3: UV-vis spectra of indigo carmine/ferric ion (2.6 μM /8.64 μM) with the increasing of GA. The concentrations of GA is 0, 3.6, 180, 216, 260, 345, 432, 518, 720, 900 μM from a to j, respectively. Inset: the photograph of indigo carmine/ferric ion in the absence (the left) and presence (the right) of GA (900 μM).

Under optimal conditions, a series of different concentration of GA were added to the indigo carmine/ferric ion sensing system and measured the UV-vis spectra of the system. As shown in Fig. 4, the absorbance of indigo carmine/ferric ion system decreases with the increasing of the concentration of GA from 3.6 μM to 900 μM . The calibration curve between the relative absorption changes of the sensing system and the

concentration of GA was obtained and it exhibited a good linear regression ($R^2=0.991$) in the range of 3.6-900 μM (Fig 4B). The limit of the detection (LOD) is 1.1 μM ($S/N=3$), much lower than that in the food samples, indicating that the method is very sensitive for the determination of GA. In addition, the analytical characteristics for GA detection of this method were compared with other reported methods and the results listed in Table-1. It is obvious that the presented colorimetric method is comparable with other techniques.

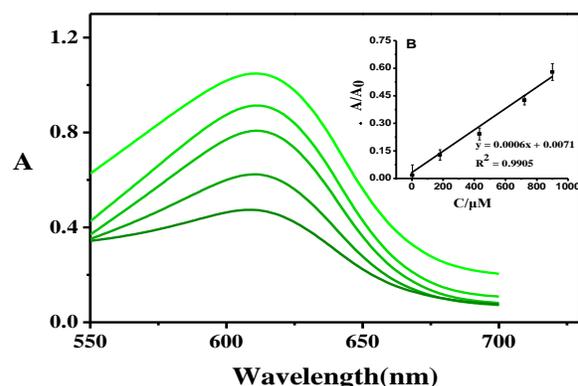


Fig. 4: Absorption spectra of indigo carmine/ Fe^{2+} sensing system in the presence of various concentration of GA: 3.6, 180, 482, 720, 900 μM .

Inset: the calibration curves of the absorption of the indigo carmine/ Fe^{2+} sensing system at 610 nm with GA the concentration from 3.6 to 900 μM .

Table-1: Comparison the current method and previous reported methods for the detection of GA.

Methods	Detection range / μM (TTX-aptamer : 5'-TTT TTA AAG TGT GCC CAC GGA GCC GAC AGG-3') (TTX-aptamer : 5'-TTT TTA AAG TGT GCC CAC GGA GCC GAC AGG-3')	LOD/ μM	Reference
Electrochemical	0.79-437.06	0.79	5
HPLC	Up to 2548.9	10.2	11
GC	Not shown	Not shown	13
FI-IR	0.23-1.07	0.199	15
Colorimetry	3.6-900	1.1	This work

Reproducibility and Specificity of the sensing system

The reproducibility of the proposed method is obtained by measuring 10 μM , 432 μM , 800 μM of GA at low, medium and high concentration level in the linear range for six times. The relative standard deviation (RSD %) values for six replicate determinations using an ultraviolet

spectrophotometer was less than 0.29%, revealing that this method has good reproducibility. In addition, the parallel determination the same sample for the detection of GA was performed and the relative standard deviation (RSD %) values for six sample determinations was less than 0.43 % for GA, revealing that this method has good precision.

In order to evaluate the selectivity of the assay, a series of other interferences which may possible coexist were explored and the results are listed in Fig 5. Only GA (0.32 mM) significantly decreased the absorption of the sensing system compared to other substances (5.4 mM) at 16-fold concentration, which reveals that this method enabled highly selective for detection of GA, over other interferences.

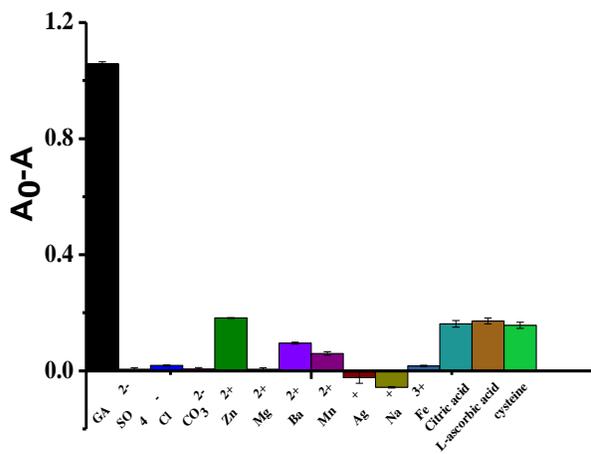


Fig. 5: Selectivity of the colorimetric assay for GA. A₀ and A are the absorption of the indigo carmine/Fe²⁺ system in the absence and presence GA or other interferences, respectively. The concentration of GA is 0.32mM and other interferences are 5.4mM, respectively.

Real sample analysis

In order to verify the ability of the sensing system in the analysis of real samples, a kind of food sample of Lactone tofu was analyzed using the proposed sensing system. The results showed the content of GA was 11.2 mM in Lactone tofu, which is in accordance with the label of the product, conforming the hygienic standard of the use of food additives (GB2760-2011), the maximum usage is

15mM of the soybean products (tofu and bean curd). The recovery studies, shown in Table-2, GA in Lactone tofu were in the range of 92.84 % to 97.02 %, exhibiting the presented sensing system is suitable for GA analysis in food samples.

Table-2: Recovery of Gluconic acid in Lactone tofu.

Spiked /mM	Detected /mM	Recover /%
0.1728	0.1677±0.02	97.02±0.06
0.1728	0.1771±0.04	96.61±0.11
0.1728	0.1668±0.03	96.52±0.10
0.2159	0.2092±0.03	96.86±0.07
0.2159	0.2060±0.03	95.37±0.08
0.2159	0.2095±0.04	97.03±0.09
0.2592	0.2406±0.04	92.84±0.08
0.2592	0.2433±0.04	93.88±0.07
0.2592	0.2509±0.03	96.81±0.06

Conclusions

In conclusion, a rapid colorimetric strategy for the determination of gluconic acid in food sample was proposed. Indigo carmine dye can be reduced by gluconic acid in the presence of ferric ion, accompanying with the color changes of the solution. A sensing system with a wide linear range of gluconic acid was presented and a low cost, simple, feasible and high sensitivity method was developed. Further. The presented strategy can be applied to test real samples and an extended application is expected to other real samples.

Acknowledgements

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